

# Notice of Allowability

Application No.

09/249,543

Examiner

William W. Moore

Applicant(s)

EVANS ET AL.

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-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to the Amendment filed 20 August 2004 and the interview conducted 27 September 2004.
2. ☒ The allowed claim(s) is/are 63, 65-80, 82-87 and 90.
3. ☐ The drawings filed on \_\_\_\_\_ are accepted by the Examiner.
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
  - a) ☐ All    b) ☐ Some\*    c) ☐ None    of the:
    1. ☐ Certified copies of the priority documents have been received.
    2. ☐ Certified copies of the priority documents have been received in Application No. \_\_\_\_\_.
    3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).
  - \* Certified copies not received: \_\_\_\_\_.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.  
**THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.**

5. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.
  6. ☐ CORRECTED DRAWINGS ( as "replacement sheets") must be submitted.
    - (a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review ( PTO-948) attached
      - 1) ☐ hereto or 2) ☐ to Paper No./Mail Date \_\_\_\_\_.
    - (b) ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date \_\_\_\_\_.
- Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
7. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

## Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3. ☐ Information Disclosure Statements (PTO-1449 or PTO/SB/08),  
Paper No./Mail Date \_\_\_\_\_
4. ☐ Examiner's Comment Regarding Requirement for Deposit  
of Biological Material
5. ☐ Notice of Informal Patent Application (PTO-152)
6. ☒ Interview Summary (PTO-413),  
Paper No./Mail Date 20040927.
7. ☒ Examiner's Amendment/Comment
8. ☒ Examiner's Statement of Reasons for Allowance
9. ☐ Other \_\_\_\_\_.

## EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Amend claims 63, 65-68, 70, 74-76, 80, 83-85 and 90 thus:

63. (Amended) A method for generating a cysteine or a selenocysteine at the N-terminus of a target protein, comprising:

expressing in a host cell, a nucleic acid encoding a fusion protein comprising an intein and a target protein wherein the ~~having an intein-encoding coding sequence is 5'-proximal to a codon specifying adjacent to a coding sequence for the a~~ cysteine or a selenocysteine at the amino terminus of in the target protein; and

cleaving the intein from the target protein so as to generate the cysteine or selenocysteine at the N-terminus of the target protein.

65. (Amended) A method for ligating target proteins, ~~the method~~ comprising the steps of:

- (a) expressing from a first plasmid in a first host cell, a first fusion protein comprising a first target protein having a C-terminus fused to an intein or modification thereof;
- (b) expressing from a second plasmid in the first host cell or a second host cell, a second fusion protein comprising a ~~the~~ second target protein having an N-terminal cysteine or selenocysteine ~~terminus~~ fused to an intein or modification thereof;
- (c) obtaining an extracellular preparation of the first fusion protein and an extracellular preparation of the second fusion protein;
- (d) adding a thiol reagent to the extracellular preparation of the first fusion protein whereby the first intein is cleaved so as to form a C-terminal thioester on the first target protein;
- (e) cleaving the second intein or modification thereof from the second target protein in the extracellular preparation of the second fusion protein and forming an N-terminal cysteine or selenocysteine on the second target protein; and

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- (f) permitting ligation of the first target protein with the C-terminal ~~e-terminal~~ thioester with the second target protein of step (e).
66. (Amended) The method of claim 65, wherein the first intein is the an Mth RIRI intein depicted in (SEQ ID NO:24), wherein or a modified form of the Mth RIRI intein is modified.
67. (Amended) The method of claim 65, wherein the second intein is the an Mth RIRI intein depicted in (SEQ ID NO:24), wherein or a modified form of the Mth RIRI intein is modified.
68. (Amended) The method of claim 66 or 67, wherein the modification of the Mth RIRI intein comprises a substitution of alanine Ala instead of for the asparagine at position 134 Asn<sup>434</sup> at the C-terminus or a substitution of alanine or serine Ala or Ser for the cysteine at position 1 instead of Cys<sup>4</sup> at the N-terminus.
70. (Amended) The method of claim 65, wherein step (c) further comprises: purifying the first or second fusion protein from the extracellular preparation, the first or second fusion protein.
74. (Amended) A method for cyclization of a target protein having an N-terminal cysteine or a selenocysteine, the method comprising the steps of:
- (a) expressing from a plasmid in a host cell, a fusion protein comprising a target protein having either a cysteine or a selenocysteine at the a C-terminus and an N-terminus, and two inteins, wherein the a first intein, or a modification thereof, is fused to at the C-terminus of the target protein and the a second intein, or modification thereof, is fused to at the N-terminal terminus adjacent to a cysteine or selenocysteine amino acid on of the target protein;
  - (b) obtaining an extracellular preparation of the expressed fusion protein;
  - (c) inducing cleavage of cleaving the fusion protein, comprising addition of a thiol reagent, to remove the first and second inteins from the target protein thereby and obtaining the target protein having a C-terminal thioester and an N-terminal cysteine or selenocysteine; and
  - (d) permitting intramolecular ligation of the N-terminus of the target protein to the C-terminus of the target protein thereby forming to form a cyclized protein.
75. (Amended) The method of claim 74, wherein the intein is an the Mth RIRI intein depicted in (SEQ ID NO:24), and the Mth RIRI is or a modified form of the Mth RIRI

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- intein wherein the modification comprises a substitution of alanine ~~Ala instead of~~ for the asparagine at position 134 ~~Asn~~<sup>134</sup> at the C-terminus or a substitution of alanine or serine ~~Ala or Ser~~ for the cysteine at position 1 ~~instead of Cys~~<sup>4</sup> at the N-terminus.
76. (Amended) The method of claim 74, wherein (i) modulation of any of temperature, pH, salt, the concentration of chaotropic agents or combinations thereof cleaves the N-terminal cysteine or selenocysteine is formed by cleavage of the intein or modification thereof from the target protein to form the N-terminal cysteine or selenocysteine, by modulating any of temperature, pH, salt, chaotropic agents or combinations thereof and (ii) the addition of the thiol reagent forms the C-terminal thioester on the target protein is formed by adding a thiol reagent.
80. (Amended) A method for cyclization of a target protein having an N-terminal cysteine or selenocysteine, comprising:
- adding a thiol reagent to a fusion protein comprising a target protein having an N-terminal cysteine or selenocysteine and (i) an intein, or modification thereof, fused to the at a C-terminus of the target protein and (ii) a cysteine or selenocysteine at an N-terminus of the target protein, in order to induce cleavage of the intein from the target protein and the formation of a C-terminal thioester on the target protein; and
- permitting intramolecular ligation of the N-terminus of the C-terminal thioester of the target protein to the N-terminal cysteine or selenocysteine of the target protein for cyclization of the target protein.
83. (Amended) The method of claim 82, wherein the first intein is the an Mth RIRI intein depicted in (SEQ ID NO:24), wherein or a modified form of the Mth RIRI intein is modified.
84. (Amended) The method of claim 82, wherein the second intein is the an Mth RIRI intein depicted in (SEQ ID NO:24), wherein or a modified form of the Mth RIRI intein is modified.
85. (Amended) The method of claim 83 or 84, wherein the modification of the Mth RIRI intein comprises a substitution of alanine ~~Ala instead of~~ for the asparagine at position 134 ~~Asn~~<sup>134</sup> at the C-terminus or a substitution of alanine or serine ~~Ala or Ser~~ for the cysteine at position 1 ~~instead of Cys~~<sup>4</sup> at the N-terminus.

90. (Amended) A modified Mth RIRI intein, wherein the intein comprising the amino acid sequence depicted in SEQ ID NO:24 comprises and the modification comprising a substitution of alanine Ala instead of for the asparagine at position 134 Asn<sup>134</sup> at the C-terminus of the intein or a substitution of alanine or serine Ala or Ser for the cysteine at position 1 instead of Cys<sup>4</sup> at the N-terminus of the intein.

Authorization for this examiner's amendment was given in a telephone interview with Dr. Harriet M. Strimpel on September 27, 2004.

The following is an examiner's statement of reasons for allowance:

The amendments to claims 63, 65, 82 and 88 filed August 27, 2004, overcome the rejections under the first paragraph of 35 U.S.C. § 112 affecting claims 63, 65-72 and 82-87 as well as rejections of record under 35 U.S.C § 102. The amendment to claim 65 also overcomes both the double-patenting rejection of claims 65 and 69-72 over claims of the '247 patent to Comb et al. and the double patenting rejection of dependent claims 66 and 67 herein over claims of Comb et al. in view of Smith et al., 1997, as well as the rejection of record under 35 U.S.C. § 103(a) based on teachings of Comb et al. The amendment to claim 73 overcomes both of the double-patenting rejections over claims of the '247 patent to Comb et al. as well as the rejection under 35 U.S.C. § 103(a) over Comb et al. in view of others. The cancellations of claims 64, 81 and 89 of August 27, 2004, render the prior art rejections of these claims moot.

Applicants arguments accompanying the amendments filed August 27, 2004, are persuasive in overcoming the enablement rejection of claims 74, 76-80, 82, 86 and 87 under 35 U.S.C. § 112, thus the examiner's amendment above restates claims 75, 83 and 84 so that they describe first and/or second inteins that may either be the Mth RIRI intein depicted in SEQ ID NO:24, or a modified form of the Mth RIRI intein. Claims 66 and 67 are similarly restated in the examiner's amendment and claims 63, 65, 68, 70, 74, 76, 80, 85 and 90 are restated in the examiner's amendment to reorganize and clarify the intended subject matter

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Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

*Conclusion*

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to William W. Moore whose telephone number is now 571.272.0933. The examiner can normally be reached between 9:00AM and 5:30PM EST. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura Achutamurthy, can now be reached at 571.272.0928. The fax phone numbers for all communications for the organization where this application or proceeding is assigned remains 703.872.9306. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is now 571.272.1600.

William W. Moore  
September 27, 2004



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